P1 1132491

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February 19, 2004

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APPLICATION NUMBER: ***60/431,937***
FILING DATE: ***December 09, 2002***
RELATED PCT APPLICATION NUMBER: *PCT/US03/39196*

By Authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

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Certifying Officer

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12-10-02 APPROV

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PTO/SB/16 (12-97)
Approved for use through 1/31/98. OMB 0651-0037
Patent and Trademark Office: U.S. DEPARTMENT OF COMMERCE

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
David	*Feifel*	*La Jolla, CA*
Gilia	*Mendez*	*San Diego, CA*
Paul	*Shilling*	*San Diego,Ca*

☐ *Additional Inventors are being named on the ___ separately numbered sheets attached hereto*

TITLE OF THE INVENTION (280 characters max)

Method of Inhibiting Neural Transmission Mediated by Serotonin and Enhancing Sensorimotor Gating

CORRESPONDENCE ADDRESS

Direct all correspondence to:

☐ Customer Number [ ] → *Type Customer Number here*

Place Customer Number Bar Code Label here

OR

☑ Firm or Individual Name	*FUESS & DAVIDENAS*				
Address	*17258 Amarillo Road*				
Address					
City	*Ramona*	State	*CA*	ZIP	*92065*
Country	*USA*	Telephone	*(760) 788-7401*	Fax	*(760) 788-5846*

ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 19 ☐ Small Entity Statement

☑ Drawing(s) *Number of Sheets* 2 ☐ Other (specify)

METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT *(check one)*

	FILING FEE AMOUNT ($)
☑ A check or money order is enclosed to cover the filing fees	
☑ The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: [ ]	*$80.00*

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government

☑ No

☐ Yes, the name of the U.S Government agency and the Government contract number are _____

Respectfully submitted, William C. Fuess

SIGNATURE _____ Date 12/09/02

TYPED or PRINTED NAME *William C. Fuess* REGISTRATION NO. *30,054* *(if appropriate)*

TELEPHONE *(760) 788-7401* Docket Number: *SD 2003-090*

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, DC 20231

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FEE TRANSMITTAL

Patent fees are subject to annual revision on October 1
These are the fees effective October 1, 1997.
Small Entity payments must be supported by a small entity statement, otherwise large entity fees must be paid. See Forms PTO/SB/09-12
See 37 C.F.R. §§ 1.27 and 1.28

TOTAL AMOUNT OF PAYMENT ($) 80.00

Complete if Known	
Application Number	
Filing Date	12/09/02
First Named Inventor	David Feifel
Examiner Name	
Group / Art Unit	
Attorney Docket No.	SD 2003-090

METHOD OF PAYMENT (check one)

1. ☐ The Commissioner is hereby authorized to charge indicated fees and credit any over payments to:

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Deposit Account Name

☐ Charge Any Additional Fee Required Under 37 C F R §§ 1 16 and 1 17
☐ Charge the Issue Fee Set in 37 C F R § 1.18 at the Mailing of the Notice of Allowance

2. ☑ **Payment Enclosed:** ☑ Check ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	790	201	395	Utility filing fee	
106	330	206	165	Design filing fee	
107	540	207	270	Plant filing fee	
108	790	208	395	Reissue filing fee	
114	150	214	75	Provisional filing fee	80.00

SUBTOTAL (1) ($) 80.00

2. EXTRA CLAIM FEES

		Extra Claims		Fee from below	=	Fee Paid
Total Claims		-20** =		X	=	
Independent Claims		- 3** =		X	=	
Multiple Dependent					=	

**or number previously paid, if greater, For Reissues, see below

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	22	203	11	Claims in excess of 20
102	82	202	41	Independent claims in excess of 3
104	270	204	135	Multiple dependent claim, if not paid
109	82	209	41	** Reissue independent claims over original patent
110	22	210	11	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 0

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet.	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	950	217	475	Extension for reply within third month	
118	1,510	218	755	Extension for reply within fourth month	
128	2,060	228	1,030	Extension for reply within fifth month	
119	310	219	155	Notice of Appeal	
120	310	220	155	Filing a brief in support of an appeal	
121	270	221	135	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,320	241	660	Petition to revive - unintentional	
142	1,320	242	660	Utility issue fee (or reissue)	
143	450	243	225	Design issue fee	
144	670	244	335	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Petitions related to provisional applications	
126	240	126	240	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	790	246	395	Filing a submission after final rejection (37 CFR 1.129(a))	
149	790	249	395	For each additional invention to be examined (37 CFR 1.129(b))	

Other fee (specify) _____
Other fee (specify) _____

* Reduced by Basic Filing Fee Paid

SUBTOTAL (3) ($) 0

SUBMITTED BY				Complete (if applicable)	
Typed or Printed Name	William C. Fuess			Reg. Number	30,054
Signature	William C. Fuess	Date	12/09/02	Deposit Account User ID	

Burden Hour Statement. This form is estimated to take 0 2 hours to complete Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS SEND TO Assistant Commissioner for Patents, Washington, DC 20231.

Brought in by: LB

CASE No. 2003-090

PROPRIETARY INFORMATION

TECHNOLOGY TRANSFER and INTELLECTUAL PROPERTY SERVICES, UCSD
INVENTION AND TECHNOLOGY DISCLOSURE FORM

A. TITLE OF INVENTION

Create a short title describing the general nature of the invention without revealing the specific details that would enable others to reproduce the invention (e g , new anticancer compound, method for chip fabrication, etc) Please limit the title to 60 characters

Method of inhibiting neural transmission mediated by serotonin-2A and enhancing sensorimotor gating.

B. UCSD INVENTOR(S)

*List all UCSD employees or students who **intellectually** contributed to the invention Please also indicate any joint or special appointment with non-UCSD institutions (e.g., VA and HHMI) in the "Position" box.*

Name: David Feifel	S[redacted]	Position: Assoc. Professor In Residence Joint or Non-UCSD Affiliation:
Dept.:Psychiatry Mail Code: MC8218	Work Address: 200 West Arbor Drive San Diego, CA 92130-8218	
Wk. Phone: (619) 543-2485	Fax: (619) 543-3738	Email: dfeifel@ucsd edu
Hm Address: 8241 La Jolla Scenic Drive North La Jolla, CA 92037		Hm Phone (858) 558-6113

Name: Gilia Mendez	SS#:	Position: Lab Assistant Joint or Non-UCSD Affiliation:
Dept.. Mail Code:	Wk. Address.	
Wk Phone:	Fax:	Email:
Hm. Address:		Hm. Phone:

Name Paul Shilling	SS#.	Position. Post-Doc Fellow Joint or Non-UCSD Affiliation:
Dept.. Mail Code:	Wk. Address.	
Wk. Phone.	Fax:	Email:

C. INVENTORS NOT AFFILIATED WITH UCSD

If an inventor is not a UCSD employee or student, please provide information below

Name:	Position: Chemist	Nature of employment:
Employer:	Wk. Address:	
Wk. Phone.	Fax:	Email.
Nature of Contribution (please provide information explaining why this person is a co-inventor)		

Name:	Position:	Nature of employment:
Employer:	Wk. Address	
Wk. Phone:	Fax:	Email:
Nature of Contribution (please provide information explaining why this person is a co-inventor):		

Please attach a separate page for additional non-UCSD inventors

D. FUNDING SOURCES FOR THE PROJECT UNDER WHICH THE INVENTION WAS MADE

As funding often carries obligations to a sponsor, be sure to include all outside agencies, organizations, or companies that provided any funding to any inventor for the research that led to the conception or first actual reduction to practice of the invention. Please include any companies that have supplied materials in exchange for intellectual property rights.

SPONSORS	GRANT NUMBER	PRINCIPAL INVESTIGATOR
National Alliance Research and Schizophrenia		David Feifel
NIH	MH6245-01 Also Supplied compound for inventive studies	David Feifel
Parke Davis	Supplied Compound for initial study	

Is there any other corporate relationship that we should be aware of concerning this invention? Y N

Name of Company: ParkeDavis

Please explain the nature of the relationship: Synthesized and patented compound

E. MATERIAL TRANSFER AGREEMENT (MTA)

Please list and attach copies, if available, of any Material Transfer Agreement or any other written or oral agreement used to obtain any material used for this invention.

An MTA was possibly executed with Parke Davis to obtain the original sample of PD149163 to do initial studies resulting in one publication (Feifel et al, 1999, Journal of Pharmaceuticals and Experimental Therapeutics). Parke Davis stopped producing this compound. Subsequently I have obtained compound from SRI Internation, Menlo Park, CA free of charge (underwritten by NIH chemical synthesis program).

F. DESCRIPTION OF EVENTS:

This information is important for deciding priority of invention and/or legal "bars" to patenting. In general, publications, in any medium, before the date a formal patent application is filed in a national patent office can cause a bar to patent filing in most foreign countries. While United States patent law allows inventors up to one year to file a patent application after the first publication, public use, or sale, the loss of foreign rights is often very important to potential industrial licensees. **SEE APPENDED SECTION BELOW**

EVENTS	DATE	INDICATE THE WRITTEN RECORD (e.g., notebook, letter, email). IF ORAL DISCLOSURE, INDICATE TO WHOM.
1. Initial conception of the idea		
2. First description of complete invention, oral or written		
3. First successful demonstration (first actual reduction to practice)		
4. Has this work been. i. submitted for publication? Y N ii. accepted for publication? Y N iii. Published? Y N		
5. Have you presented this work at a conference or meeting? i. Did you submit an abstract? Y N ii. Was abstract published? Y N iii Name of conference or meeting? Y N i. Did presentation include handouts? Y N		

G. INVENTORS' SIGNATURES

By signature below, I acknowledge my responsibilities and rights to royalty-sharing under the current University of California Patent Policy.

David Thiel — 12/3/02
Inventor signature — Date

Inventor signature — Date

H. WITNESS - invention disclosed to and understood by:

12-3-02
Witness signature — Date

LARRY BRAND
Print witness name

I. ABOUT THE INVENTION

Please write a summary of the invention following the numbered guidelines listed below Since this information will be used to determine patentability, commercial uses, and potential licensees of the invention, please provide as many details as possible. If you have a written manuscript descriptive of your invention, please also attach a copy to this form.

1. What exactly does your invention do?

My invention is that neurotensin peptide mimetics modified to enter the CNS (PD149163) do the following:

Reverses sensorimotor gating deficits (as measured by prepulse inhibition of startle reflex) in Brattleboro Rat, a commercial rat with a single gene deficit which I discovered to has innate abnormalities in prepulse inhibtion similar to humans with neuropsychiatric disorders (i e schizophrenia, huntingtons disease, bipolar disorder) This effect is likely predictive of therapeutic effects in neuropyshiatric disorders.

Also improves sensorimotor gating in normal rats.

Also reverses deficits in sensorimotor gating deficits produced by drugs that are serotonin-2 agonists and alpha-1 agonists (previous pharmacological effects not attributed to neurotensin or neurotensin drugs).

2. What is unique, novel, or better about your invention as compared to existing art?

PD149163 is appears to be more potent and longer lasting than other psychotropic drugs tested in preclinical tests. It performed better than current "gold standard" antispychotic drugs and evidence of effects lasted several days after a single administration which is not seen with currently existing psychotropic drugs.

Compared to existing psychotropic medication:
-Appears to have unique mechanism of action (i.e. modulates relevant CNS circuits implicated in neuropsychiatric conditions and normal sensorimotor gating without directly bind to the either monoamine, GABA, glutamate or acetylcholine receptors (the therapeutic mechanism attributed to all current psychotropic medication).
-appears to have a favorable side effect profile base upon preclinical tests
-Appears to have longer duration of effects from single administration of other psychotropic drugs tested

-Compared to existing art regarding this category of compound (neurotensin agonist), it appears to have previously unknown mechanisms and effects that I discovered (i.e. antagonism of serotonin-2A and alpha 1-adrenergic functions and facilitation of sensorimotor gating in animals with genetically induced deficient sensorimotor gating and in normal animals) Furthermore neurotensin analogs

3. What is the existing art to which you are comparing?

1. Antipsychotic drugs
2. Antidepressant Drugs
3. Neurotensin mimetics (e g what is currently known about this same compound, and family and other neur that enter the CNS e.g NT69L)

4. Describe how your invention works (or may work). Please include drawings, schematics, figures, etc., necessary to explain how the invention works or may work

Mechanism is not fully elucidated.

However, in preclinical predictive test of potential psychotropic (especially antipscyhotic) usefuleness a representative compound of this family (PD149163) was able to reverse startle abnormalities (sensorimotor gating, prepulse inhibition) in genetically mutant rats more effectively than some of the best available antipsychotics. It was also able to improve startle parameters in normal rats Therefore it appears to modulate sensorimotor gating circuits

In other preclinical tests done in my laboratory aimed at elucidating mechanism, it appeared to reverse CNS effects of a serotonin 5HT2A and alpha-1 adrenergic agonists, suggesting inhiition of neurotransmission mediated by these receptors. (Previously inhibition of dopamine receptor mediated neurotransmission was the only therapeutic mechanism associated with this family of compounds)

5. Describe the stage of development of the invention (e.g., concept stage, experimental data stage, computer model simulation stage, working prototype stage, etc.). Please include data, photographs, etc., indicating the stages of development.

Preclinical Stage.

Test in my laboratory using animal tests predictive of psychotropic efficacy. Some of these predictive tests have been established in the field, others I have innovated.

6. What are potential commercial applications of your invention?

Drugs that target neurotensin receptors that may have therapeutic effects for neuropsychiatric disorders: Including. schizophrenia, schizoaffective disorder, bipolar disorder, depression, anxiety disorders. autism, Huntingtons disease, obsessive-compulsive disorders.
May also have prophylactic efficacy for people at high risk for these conditions.
May also act as an extended duration therapy for neuropsychiatric disorders
May work more rapidly than current drugs which all have a delayed effect (faster effect in preclinical studies)
May augment the effects of current psychotropic drugs when used in combination.
May also improve cognitive performance in people who do not have neuropsychiatric disorders

J. COMPANIES

Based on your knowledge, please provide the names and addresses of companies that are, or may be, interested in manufacturing, using, and/or further developing your invention.

Company & Contact Name	Company Address
Roche, Janssen, Eli Lilly, Pfizer, Bristol Myers Squibb, AstraZeneca, Pharmacy and Upjohn, Parke-Davis	
Neurocrine, Solvay, Abbot Laboratories	

1. Please list or attach any literature references that most closely describe the state of the related art before your invention. If possible, please consider doing a search of the literature, because it will help in the evaluation of your invention.

U.S. Patent # 5,393, 740 "Neurotensin Hexapeptides" (02-28-95)
U S. Patent # 5,407, 916 "Neurotensin Mimetics as Central Nervous System Agents" (04-18-1995)
Kinkead B. et al "Neurotensin an endogenous Antipsychotic? Curr Opin Pharmacol, 2002 Feb: 2(1):99-103
Feifel et al, 1999 "Novel Antipsychotic-Like Effects On Prepulse Inhibition of Startle Produced By A Neurotensin Agonist. (Appended)
Feifel et al., (In Press) A systemically administered neurotensin agonist blocks disruption of prepulse inhibition produced by a serotonin-2A agonist. (Appended).
Feifel et al, 2002 (Abstract of poster to be presented at ACNP meeting, December 9, 2002) EVIDENCE THAT NON-DOPAMINE MECHANISMS CONTRIBUTE TO THE ANTIPSYCHOTIC-LIKE EFFECTS OF NEUROTENSIN AGONISTS (Appended)

2. If a company is interested in licensing your invention, would you be interested in assisting or working with the company to develop your invention into a product under a sponsored or research agreement with the University? Y / N

YES
I would also seriously consider establishing a company to acquire license and further develop invention.

Events relevant to invention:

Preclinical work suggest that neurotensin, an endogenous neuropeptide that has 13 amino acide may have use as a antipsychotic based upon the fact that it inhibits effects of dopamine in brain. – Series of studies, multiple investigators beginning in 1980s

Parke Davis reports new family of compounds that are active fragment of neurotensin NT(8-13) chemically modified to increase stability and cross into the CNS after systemic injection (Wustrow et al, 1995) Patent filed 1995 ("Neurotensin Mimetics as central Nervous System Agents), suggesting these compounds may be effective for schizophrenia or analgesia

I collaborate with Parke-Davis investigators who supply me with an amount of one of the neurotensin mimetics (PD149163) to test it in startle-based animal screen for antipsychotic mechanisms. Results show equivocal effects c dopamine (previously implicated basis for antipsychosis) but indirect evidence for non-dopamine mechanisms of antipsychosis (reversal of the effects of glutamate antagonist). Results published in 1999 (Feifel et al, 1999)

I begin further studies on PD149163 based upon interesting results of 1999 paper. Parke Davis stopped producing compund. Therefore I have since obtained compound free of charge from SRI international which is commissioned by NIH (chemical synthesis program) to synthesis this for research purposes.

Recent studies in my lab show PD149163 reverses disruption of prepulse inhibition (a measure of sensorimotor gating) produced by a serotonin-2A receptor agonist (DOI). This suggest PD149163 (and presumably others neurotensin mimetics in this family) can antagonize the brain pathways mediated by serotonin-2A receptors. This is an important pharmacological mechanism shared by many new generation psychotropics that are useful to treat psychosis, depession, mania, anxiety Those drugs do so by directly binding serotonin-2A receptors (Neurotensin mimetics do not) This study was performed January, 2001 This finding has been accepted for publication in the journal Neuropsychopharmacology and the MS form has been placed upon their website since November, 2002 (But not formally published in print as a final document). (See attached manuscript, Feifel, Mendez and Shilling).

Recent studies in my lab with another NT69L, another NT(8-13) mimetic stabilized to enter the CNS by another chemical means (obtained by a colleague who synthesized it a Mayo in Jacksonville, Florida) did not show the ability to reverse effects of DOI (unpublished finding) Therefore, this pharmacological property may be unique to PD14916 or chemically related neurotensin mimetics (stabilized by an amide bond)

Recently a study in my lab showed that PD149163 reverses disruption of prepulse inhibition (a measure of sensorimotor gating) produced by an alpha-1 adrenergic receptor agonist (cirazoline). This suggest PD149163 (and presumably others neurotensin mimetics in this family) can antagonize the brain pathways mediated by alpha-1 adrenergic receptors. This is an important pharmacological mechanism of of many new generation antipsychotics are thought to contribute to their antipscyhotic effects (although less established than serotonin-2A inhibition). Study done in October, 2002 (unpublished).

I recently discovered that well known a genetically mutated rat (Brattleboro Rat) has a deficits in sensorimotor gating (measured as prepulse inhibition of strartle response) similar to patients with some neurospychiatric disorders. Reversal of this abnormality is likely a marker for psychotropic drugs (especially antipscyhotics)

Acute administration of PD149163 reverses this abnormality in Brattleboro rats but standard antipscyhotic drugs did not. Furthermore, PD149163 did increased baseline sensorimotor gating in normal rats. Critical study conducted August, 2002 **(unpublished but this data will be presented at the American College of Neuropharmacology meeting on December 9, 2003 as a poster)**

All these things suggest that Neurotensin agonists (or at least PD149163 and chemically related drugs) and psychotropic uses and mechanisms not previously taught by the existing art

Please send completed forms and supporting materials to.

ANTIPSYCHOTIC-LIKE EFFECTS OF NEUROTENSIN AGONISTS

Feifel, David; Melendez, Gilia; Shilling, Paul D.
Department of Psychiatry, University of California, San Diego

ABSTRACT

Neurotensin is a neuropeptide that has generated significant interest as a potential antipsychotic drug. This interest is derived from evidence that neurotensin inhibits D2 receptor transmission in the mesolimbic pathway. However, accumulating results from our laboratory using prepulse inhibition (PPI) paradigms that are predictive of antipsychotic activity, indicate that non-D2 mechanisms may contribute to the antipsychotic properties of neurotensin agonists. Specifically, we have shown that acute systemic administration of the neurotensin agonist PD149163 attenuates or blocks the PPI deficits exhibited spontaneously by Brattleboro rats and PPI deficits induced by the NMDA antagonist, dizocilpine (MK801), and the 5HT1A agonist, DOI. None of these effects are produced by antipsychotic drugs which selectively block D2 receptors. Therefore, these effects of PD149163 cannot be mediated by inhibition of D2 transmission. However, similar effects in each of these PPI paradigms are produced by atypical antipsychotic drugs that produce both D2 and 5-HT2A antagonism. Therefore, these results suggest that in addition to D2/neurotensin agonists like PD149163 may also modulate 5-HT2A transmission. Since neurotensin does not have known affinity for D2 or 5-HT2 receptors, other mechanisms must be involved. Potential mechanisms mediating these effects will be discussed. Further Current studies using other neurotensin agonists and other methodologies are underway in our laboratory and preliminary findings will be presented.

BACKGROUND

Neurotensin is a neuropeptide that exists in the mesolimbic dopamine pathway and appears to produce an inhibitory modulation of dopamine functions. Although the mechanism of this inhibitory modulation is not established, evidence suggests that activation of NT receptors reduces the affinity of D2 receptors for dopamine(1). Based upon this it has been proposed that drugs which target neurotensin receptors may have utility as antipsychotic drugs. Preclinical findings that neurotensin and neurotensin analogues block the behavioral effects of dopamine agonists support this concept(2).

Our laboratory has been studying NT analogues that have been designed to cross the blood brain barrier after systemic administration. One such analogue is PD149163, a NT(8-13) fragment modified to make it more resistant to enzymatic degradation(3). We have studied PD149163 in several animal models predictive of antipsychotic activity, which are based upon prepulse inhibition (PPI) of the startle reflex.

PPI refers to the normal decrease in startle produced when the startling stimulus is preceded by a milder stimulus (prepulse). PPI, which is a operational measure of sensorimotor gating is deficient in schizophrenia patients. Similar PPI deficits can be induced in rats by administration of certain psychotomimetic drugs such as amphetamine, PCP, or DOI (4). In this poster, we summarize findings from our labs that strongly suggest that PD149163 produces antipsychotic-like effects by non-dopamine mechanisms.

EXPERIMENT 1

I. PD149163 attenuates PPI disruption produced by a non-competitive NMDA antagonist

INTRODUCTION: Disruption of PPI produced by non-competitive NMDA antagonists such as PCP and dizocilpine are not blocked by acute administration of haloperidol and are therefore not mediated by D2 mechanisms. In contrast atypical antipsychotics which have notable 5HT2 antagonism partially attenuate disruption of NMDA antagonists (refs).

METHODS: Thirty four Sprague Dawley rats (n=8-9) received one of four doses of PD149163 (0, 0.08, 0.25, or 1 mg/kg) administered s.c. and dissolved in 0.9% saline (volume=1 ml/kg) immediately after this injection, animals received a second s.c. injection consisting of dizocilpine (0.1 mg/kg) or saline. Ten minutes after this second injection, animals were placed in separate startle chambers (SR-LAB; San Diego Instruments, San Diego, CA).

RESULTS: PD149163 antagonized dizocilpine-induced disruption of PPI.

Significant differences from the rats treated with same dose of PD149163 but no active psychotomimetic (saline) are represented by ** $P < 0.01$. Significant differences from the 0 mg/kg PD149163 condition are represented by the + symbol, $P < 0.05$.

CONCLUSION: This study shows that PD149163 is more similar to atypical antipsychotics than to typical antipsychotics such as haloperidol.

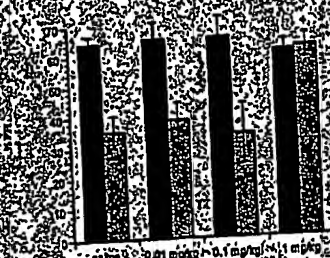
EXPERIMENT 2

II. PD149163 blocks PPI disruption produced by the 5HT2A agonist DOI.

INTRODUCTION: Disruption of PPI by DOI is not effectively blocked by haloperidol. In contrast drugs that block 5HT2A function, including atypical antipsychotics and selective 5HT2A antagonists like MDL 100109 (7), are able to reverse DOI-induced disruption of PPI.

METHODS: Thirty four Sprague Dawley rats (n=8-9) received one of four doses of PD149163 (0, 0.01, 0.1, or 1 mg/kg) administered s.c. and dissolved in 0.9% saline (volume=1 ml/kg). Thirty minutes later, animals received a second s.c. injection consisting of DOI (0.5 mg/kg) or saline. Twenty minutes later, animals were placed in the startle chambers. After one week, animals were tested a second time during which treatment and testing procedures were the same except that rats that received DOI on the first test day received saline on the second test day and vice versa.

RESULTS: PD149163 blocked DOI-induced disruption of PPI.



Significant differences from the rats treated with same dose of PD149163 but no DOI are represented by * $P < 0.05$. Significant differences from the 0 mg/kg dose of PD149163 and the same DOI treatment are represented by ** $P < 0.01$.

EXPERIMENT 3

III. PD149163 but not haloperidol reverses PPI deficits in Brattleboro rats

INTRODUCTION: Brattleboro rats are a strain of Long Evans rats with a single gene mutation causing a deficit in vasopressin production. Brattleboro rats exhibit low PPI that can be reversed by chronic but not acute treatment of haloperidol (Refs). Thus the Brattleboro rat is a potential genetic model of PPI deficits that may have predictive validity for antipsychotic drugs.

METHODS: In the PD experiment, 43 Long Evans rats and 33 Brattleboro rats received one of four doses of PD149163 (0, 1, 2, or 3 mg/kg) administered s.c. and dissolved in 0.9% saline (volume=1 ml/kg). Thirty minutes later, animals were placed in the startle chambers. In the haloperidol experiment, 50 Long Evans rats and 50 Brattleboro rats received one of four doses of haloperidol (0, 0.1, 0.5, or 1 mg/kg) administered s.c. and dissolved in distilled water (volume=1 ml/kg). Thirty minutes later, animals were placed in the startle chambers.

RESULTS: PD149163 but not haloperidol reversed PPI deficits in Brattleboro rats.

* represents significant difference between PPI average of Brattleboro rats and Long Evans rats at each corresponding drug dose. + represents significant greater PPI average compared to rats receiving 0 mg/kg dose of drug.

CONCLUSION: The fact that PD but not haloperidol was effective in this study suggests that PD produces antipsychotic-like effects on PPI by mechanisms other than D2 blockade.

GENERAL CONCLUSIONS

Much interest has been generated about the antipsychotic potential of neurotensin and neurotensin analogues. This interest has, until now, been based exclusively upon evidence that neurotensin indirectly decreases D2 transmission. Were this the case NT agonists such as PD149163 would be expected to produce effects that, at best, replicated the effects of haloperidol. However, emerging evidence from our laboratory strongly suggests that PD149163 produces antipsychotic-like effect that go beyond the effects of haloperidol and therefore are likely to be mediated by modulation of non-dopamine mechanisms. Inhibition of 5HT2 mediated pathways seems to be a likely contributor to these non-dopaminergic antipsychotic effects. Given the fact that available antipsychotic treatments have evolved beyond simple D2 antagonism, these findings are encouraging that neurotensin remains a viable target for drugs which may have clinical utility as antipsychotics.

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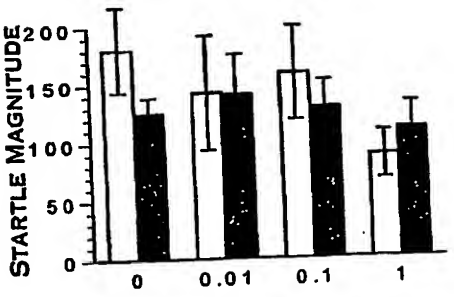
STARTLE MAGNITUDE
200
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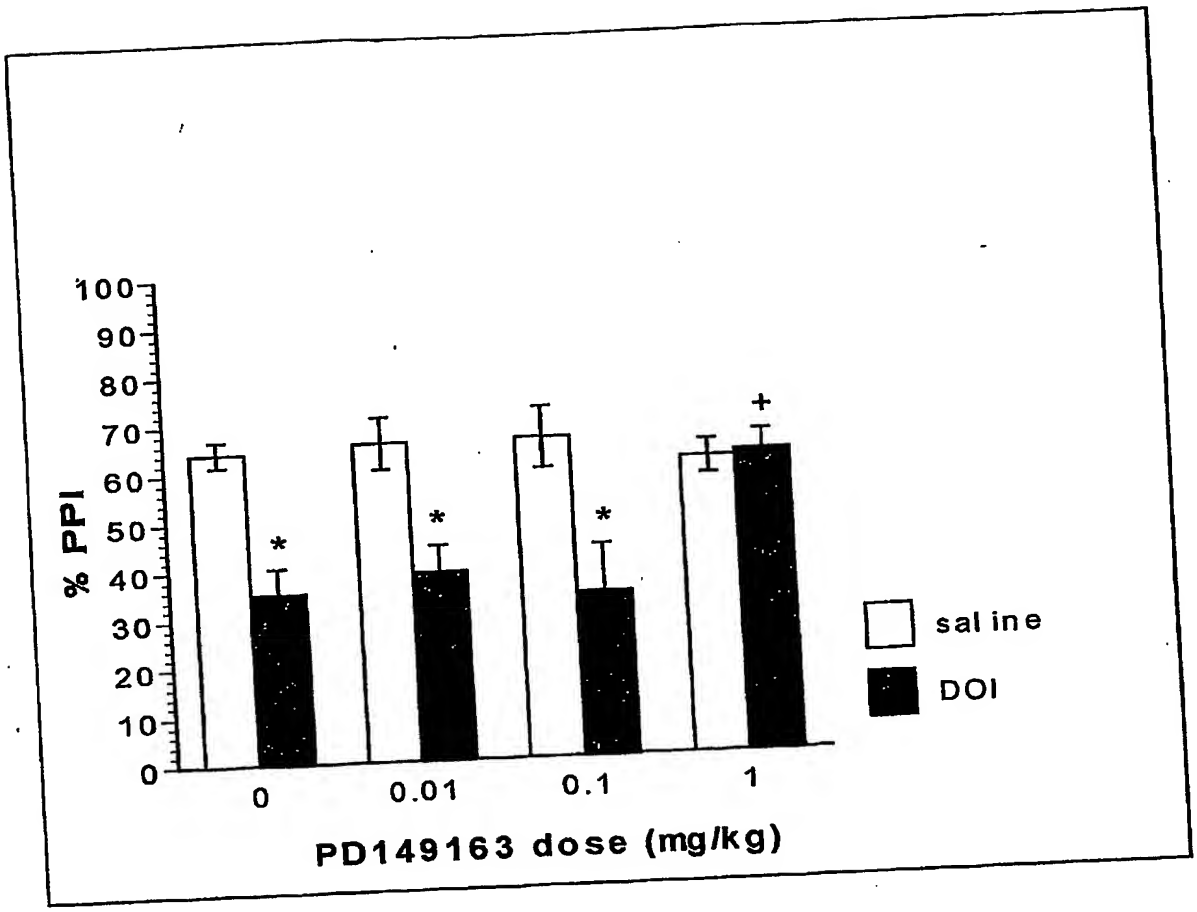
100
90
80
70
60
50
40
30
20
10
0
% PPI
*
*
*
+
0
0.01
0.1
1
PD149163 dose (mg/kg)
saline
DOI

A SYSTEMICALLY ADMINISTERED NEUROTENSIN AGONIST BLOCKS DISRUPTION OF PREPULSE INHBITION PRODUCED BY A SEROTONIN-2A AGONIST

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BRIEF REPORT

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ABSTRACT

Prepulse inhibition (PPI) of the startle reflex can be disrupted by drugs that act as agonists at the serotonin (5HT) 2A receptor, such as DOI, and this effect is blocked by drugs that inhibit 5-HT2A transmission. We tested the effects of systemic administration of PD149163, a neurotensin agonist, on DOI-induced disruption of PPI in Sprague-Dawley rats. PD149163 completely and dose-dependently blocked the PPI deficits produced by DOI. These findings suggest that, in addition to their established ability to inhibit dopamine transmission, neurotensin **agonists may also inhibit 5-HT2A transmission, a pharmacological feature associated with atypical antipsychotic drugs.**

INTRODUCTION

Prepulse inhibition of the acoustic startle reflex (PPI) is the reduction in the startle response when the startle-eliciting stimulus is immediately preceded by a weak stimulus. PPI, an operational measure of sensorimotor gating, is deficient in schizophrenia patients (Geyer et al., 2001).

Deficits in PPI can be produced in rats by a number of pharmacologically distinct "psychotomimetic" compounds (Geyer, Krebs-Thomson et al., 2001 for review). typical and atypical antipscyhotics reverse PPI deficits produced by

dopamine agonists such as amphetamine and apomorphine. The mechanism implicated has been blockade of dopamine-2 (D2) receptors. In contrast, PPI disruption produced by non-competitive NMDA antagonists such as phencyclidine (PCP) or dizocilpine is antagonized by atypical but not typical antipsychotics and blockade of serotonin (5-HT) 2A and/or alpha-1 noradrenergic, but not D2 receptors, has been implicated in this effect. Disruption by the selective 5-HT2A agonist and hallucinogen, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride (DOI) is also preferentially blocked by atypical antipsychotics and the mechanism implicated is blockade of 5-HT2A receptors since a 5-HT2A antagonist but neither a 5-HT2C antagonist nor haloperidol effectively block DOI's effect on PPI (Geyer, Krebs-Thomson et al., 2001).

The neuropeptide neurotensin appears to inhibit dopamine function and produce preclinical effects similar to antipsychotics (Kinkead and Nemeroff, 2002) suggesting that neurotensin agonists may have potential as antipsychotic drugs. Supporting this notion, we have reported that PD149163, a modified neurotensin (8-13) analog (Lys(CH2NH)Lys-Pro-Trp-tLe-Leu-OEt) (Wustrow et al., 1995) that crosses the blood brain barrier, antagonizes amphetamine-induced disruption of PPI after systemic administration (Feifel, Reza et al. 1999). In addition, we found that PD149163 antagonizes PPI deficits produced by the non-competitive NMDA antagonist dizocilpine (Feifel, Reza et al. 1999), suggesting that this compound modulates 5-HT2A and/or alpha-1 adrenergic transmission (Geyer, Krebs-Thomson et al., 2001). There is evidence that neurotensin regulates 5-HT brain systems (Heaulme, Leyris, et al., 1998) therefore, we hypothesized that PD149163 may produce antagonism of dizocilpine-

induced disruption of PPI by blockade of 5-HT2A transmission. In order to test this hypothesis we investigated whether PD149163 could antagonize PPI disruption produced by DOI, a selective 5-HT2A agonist.

METHODS

All experimental procedures were conducted in accordance with the University of California, San Diego guidelines for animal care and experimentation. Thirty-four male Sprague Dawley rats (250-300 grams at testing, Harlan Laboratories, San Diego) were housed **under a 12h:12h light:dark schedule.** On test days they were administered subcutaneous (SC) injections of 0 (saline), 0.01, 0.1, or 1 mg/kg of PD149163 (**SRI International, Menlo Park, CA**). Thirty minutes later they were injected SC with either saline or 0.5 mg/kg DOI (Sigma Chemicals, St. Louis, Mo). Animals were tested in startle chambers (San Diego Instruments, San Diego, CA) 20 minutes later. After one week, animals were tested a second time during which treatment and testing procedures were the same except that rats that received DOI on the first test day received saline on the second test day and vice versa. **All testing occurred during the light phase of the rats' circadian illumination schedule.**

Once placed in startle chambers each rats had a 5-minute acclimation period. A 65-dB background noise was continuously present throughout the session. The acclimation was followed by a 15-minute PPI test session during which rats were presented with 40-msec 120 dB startle pulses without a prepulse, or pulses preceded 100-msec by a prepulse of either 4, 8 or 12 dB above background. These four types of active

stimuli were presented in pseudorandom order along with no-sound trials with an average of 15 seconds separating them.

A startle response was recorded for all stimuli presentations. PPI for each animal was calculated as a percentage of the pulse-alone startle magnitude using the following formula: [1- (startle magnitude after prepulse-pulse pair/startle magnitude after pulse only] X 100. PPI data was analyzed using a repeated measures ANOVA with PD149163 dose as a between-subject factor and DOI treatment and prepulse intensity as within-subject factors. Significant effects were followed by post-hoc pair-wise comparisons of individual treatment groups using Bonferroni corrected t-tests.

RESULTS

There was no main effect of PD149163 but there was a significant main effect of DOI as it significantly disrupted PPI ($F[1,30] = 56.1$, $p \leq 0.001$). There was a significant main effect of prepulse intensity on percent PPI, reflected in more intense prepulses producing greater PPI ($F[2,60] = 80.3$, $p < 0.0001$). However, there was not a significant prepulse x DOI interaction, or prepulse x PD149163 interaction or prepulse x DOI x PD149163 interaction. **Therefore, the PPI data presented in the figure (main graph) is the mean of the PPI values produced by each of the individual prepulse intensities.** There was a significant DOI x PD149163 interaction ($F[3,30] = 7.3$, $p = .001$) and the data revealed that the highest dose of PD149163 reversed the DOI-induced disruption of PPI. **Compared to rats that did not receive DOI (saline), DOI treated rats had significantly decreased PPI in the group that did not receive PD149163 (saline) ($P < 0.01$) and in the groups that received 0.01 mg/kg**

(P < 0.001) and 0.1 mg/kg (P < 0.05), but not 1 mg/kg PD149163. PPI exhibited by rats receiving DOI and 1 mg/kg PD149163 were significantly greater than PPI exhibited by rats receiving DOI and saline (P < 0.01).

There were no significant main or interaction effects of DOI or PD149163 on startle magnitude (Figure Insert).

DISCUSSION

To date, all drugs that have demonstrated a robust ability to antagonize DOI-induced disruption of PPI have been compounds with potent 5-HT2A antagonism, including the atypical antipsychotic, risperidone and the selective 5-HT2A antagonists, MDL100907 and ketanserin. In contrast, drugs that are not strong 5-HT2A antagonists, including haloperidol, the selective D2 antagonist raclopride, the 5-HT2C antagonist, SDZ SER-082, and the beta-adrenergic /5-HT1 receptor antagonist, propanolol, have failed to exhibit robust antagonism of DOI's effects on PPI (Geyer, Krebs-Thomson et al., 2001). In this respect, PD149163's ability to block DOI-induced disruption of PPI but not affect baseline PPI or startle magnitude **supports our hypothesis that PD149163 may antagonize 5-HT2A transmission.**

The modulation of 5-HT systems by neurotensin has not been extensively studied, however neurotensin has been shown to stimulate the release of 5-HT in the brain (Heaulme, Leyris, et al., 1998). Neither neurotensin nor PD149163 is known to have a strong affinity for 5-HT2A receptors. These compounds could alter 5-HT2A transmission via activation of neurotensin receptors that, in turn, modulate

5-HT2A transmission at the receptor level or further downstream. Evidence suggests that DOI-induced disruption of PPI is mediated by 5-HT2A receptors in the ventral pallidum (Sipes and Geyer, 1997), a site where neurotensin receptors have been localized (Alexander and Leeman, 1997). The current evidence that PD149163 modulates 5-HT2A transmission is notable since previous interest in neurotensin agonists as potential antipsychotics was based exclusively on evidence that they inhibited dopamine transmission. Inhibition of both 5-HT2A and D2 transmission is considered the pharmacological profile that distinguishes atypical antipsychotics from the more D2 selective typical antipsychotics **(Meltzer, 1999).**

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Figure Caption:

The effect of PD149163 on PPI (main) and startle magnitude (insert) in rats receiving saline or DOI. **PPI data represents average of PPI produced by three different prepulse intensities.** * represents significantly lower ($P<0.05$) than corresponding non-DOI treatment. + represent significantly greater ($P<0.01$) than rats receiving 0 mg/kg dose of PD149163 and the same DOI treatment.

Brought in by: LB

CASE No. 2003-091

PROPRIETARY INFORMATION

TECHNOLOGY TRANSFER and INTELLECTUAL PROPERTY SERVICES, UCSD
INVENTION AND TECHNOLOGY DISCLOSURE FORM

A. TITLE OF INVENTION

Create a short title describing the general nature of the invention without revealing the specific details that would enable others to reproduce the invention (e.g., new anticancer compound, method for chip fabrication, etc.). Please limit the title to 60 characters.

A method of screening for therapies for neuropsychiatric disorders using a Norway Rat Model

B. UCSD INVENTOR(S)

*List all UCSD employees or students who **intellectually** contributed to the invention. Please also indicate any joint or special appointment with non-UCSD institutions (e.g., VA and HHMI) in the "Position" box.*

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Wk. Phone:	**Fax:**	**Email:**
Hm. Address:		**Hm. Phone:**

Name:	**SS#:**	**Position: Post-Doc Fellow** **Joint or Non-UCSD Affiliation:**
Dept.: **Mail Code:**	**Wk. Address:**	
Wk. Phone:	**Fax:**	**Email:**

I. ABOUT THE INVENTION

Please write a summary of the invention following the numbered guidelines listed below. Since this information will be used to determine patentability, commercial uses, and potential licensees of the invention, please provide as many details as possible. If you have a written manuscript descriptive of your invention, please also attach a copy to this form.

1. What exactly does your invention do?

Represents a model which provides preclinical predictive information of whether a treatment is likely to be useful as psychotropic drug, particularly an antipsychotic.

2. What is unique, novel, or better about your invention as compared to existing art?

Can distinguish between typical (first generation) and "atypical" (newer generation) antipsychotic drugs.

Does not require manipulation (e.g. drug, surgical or environmental) of animals before examining test compound, as do existing in-vivo models.

Has stronger cross-species homology to humans.

Can provide comparative information about for a test compound regarding potency, speed of onset and duration of therapeutic-like effects.

3. What is the existing art to which you are comparing?

1. Existing in-vivo predictive models of antipsychotic effects (e.g. reversal of effects of social isolation, psychomimetic drugs, CNS lesions)
2. Existing ex-vivo predictive models of antipsychotic effects (e.g. affinity assays, electrophysiology, gene expression)

4. Describe how your invention works (or may work). Please include drawings, schematics, figures, etc., necessary to explain how the invention works or may work.

Brown Norway (BB) rats are administered a putative psychotropic treatment (e.g. several doses of a test drug) and then subsequently tested in startle chambers. Prepulse inhibition (PPI) of their startle reflex is measured. Normally BN rats have PPI levels that are low compared to other rat strains such as the Kyoto Wistar (the genetically closest animal). Treatments that fully or partially increase levels of PPI in BB rats to the levels of normal rats (e.g. Wistar Kyoto) have strong clinical potential as psychotropic drugs (e.g. antipsychotic potential).

5. Describe the stage of development of the invention (e.g., concept stage, experimental data stage, computer model simulation stage, working prototype stage, etc.). Please include data, photographs, etc., indicating the stages of development.

Conceptual with part experimental validation

6. What are potential commercial applications of your invention?

Screening new therapies for neuropsychiatric disorders.

CLAIMS

What is claimed is:

1. A model for providing preclinical predictive information concerning effectiveness of a treatment for neuropsychiatric disorders, comprising:

administering to a rat a particular therapy such as a pharmaceutical preparation containing a putative psychotropic drug or manipulation; and

measuring prepulse inhibition in a startle reflex response chamber

wherein an increased prepulse inhibition level indicates a strong clinical potential as a psychotropic drug.

2. The model according to claim 1, wherein the rat is a Norway rat.

3. A method for screening new therapies for neuropsychiatric disorders, comprising:

administering to a rat a particular therapy such as a pharmaceutical preparation containing a putative psychotropic drug or manipulation; and

measuring prepulse inhibition in a startle reflex response chamber

wherein an increased prepulse inhibition level indicates a potentially effective clinical treatment.

4. The method according to claim 3, wherein the rat is a Norway rat.

5. The method according to claim 3, wherein the neuropsychiatric disorder is schizophrenia.

6. The method according to claim 3, wherein the neuropsychiatric disorder is Huntington's Disease.

7. The method according to claim 3, wherein the neuropsychiatric disorder is bipolar disorder.